

Supplementary Material 2 (Ross Alexander, Martin Kos)

RiboSys reporter sequences

Reporter constructs are as in Parker and Hilleren (2003; Mol Cell **12**, 1453-1465), with the addition of two λ boxB and six MS2 repeat sequences as shown below.

Sequence of Ribo1:

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1  GAATTCGAA AATTTACTGA ATTAACAATG TCTTTATCTT CAAAGATCTG
51  GATTCTGGTA TGTTC TAGCG CTTGCACCAT CCCATTTAAC TGTAAGAAGA
101 ATTGCACGGT CCCAATTGCTCGATGGGCCCTGAAGAAGGGCCCTAATCTC
151 GATGGGCCCTGAAGAAGGGCCCTAATCTCGAGAGATT CTCTTTTACC TTT
201 TTTTACTATTTTCACT CTCCATAAC CTCCTATATT GACTGATCTG TAA
251 TAACCACGATATTATTG GAATAAATAG GGGCTTGAAA TTTGGAAAAA AAA
301 AAAAAGTGAATATTTT CGTGATAAGT GATAGTGATA TTCTTCTTTT ATT
351 TGCTACTGTTACTAAGT CTCATGTACT AACATCGATT GCTTCATTCTTTT
401 TGTGCTATATTATATGTTTATCTCC CATGTCTCTA CTGGTGGTGG TG
451 CTTCTTTGGAATTATTGG AAGGTAAGGA ATTGCCAGGT GTAGCTTTCT TA
501 TCCGAAAAGAAAAGTAGT ttaattaaCA TCTTTTACCC ATACGATGTT CC
551 TGAATATGCGGGCTATCC CTATGACGTC CCGGACTATG CAGGATCCTA TCC
601 ATATGACGTTCAGATT ACGCTGCTCA GTGCTga ggc ggcgcATTGA ATT
651 GAATTGAAATCGACCTA GCTAGCAGATCCTAAGGTACCTAATTGCCTAGA
701 AAACATGAGGATCACCCATGTCTGCAGGTCGACTCTAGAAAACATGAGGA
751 TCACCCATGTCTGCAGTATTCCCGGGTTCATTAGATCCTAAGGTACCTAA
801 TTGCCTAGAAAACATGAGGATCACCCATGTCTGCAGGTCGACTCTAGAAAA
851 CATGAGGATCACCCATGTCTGCAGTATTCCCGGGTTCATTAGATCCTAAG
901 GTACCTAATTGCCTAGAAAACATGAGGATCACCCATGTCTGCAGGTCGAC
951 TCCAGAAAACATGAGGATCACCCATGTCTGCAGTATTCCCGGGTTCATTA
1001 GATCCCTAGCTAGCATTCGAT AGATCAATTT TTTCTTTTCTCTTC
1051 CCCATCCTTACGCTAAAATAATAG TTTATTTTAT TTTTGAATATTTT
1101 TATTTATATACGTATATATAGACTA TTATTTATCT TTTAAGATTATTAAG
1151 ATTTTATTAATAAAAAA AAATTCGCCCC TTTAATGCC TTTATGCAGT TTTT
1201 TTCCCATTCGATATTTCTATGTCGG GTCAGCGTAT TTTAAGTTTA ATAA
1251 CTCGAA AATTCTGCGTTCGTTAGCGGCCGC
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Explanation of reporter gene components:

ACT1 5' UTR (21 bases)

First exon = PGK1/ ACT1 fusion

ACT1 intron (58 to 422) containing two copies of λ boxB sequence (120 to 177)

Second exon = mini PGK1

3 copies of HA (527 to 632)

6 copies of MS2

PGK1 3'UTR with mapped 3' end cleavage sites underlined and italicised

Useful restriction sites are underlined

Mutant variants (sequence changes shown bold and italicised above):

5'SSRibo1: 5' splice site (58) mutated to C.

BSRibo1: T at (378) mutated to A and cryptic branchsite ACTAAGT removed.

3'SSRibo1: 3' splice site (422) mutated to C.

Sequence of intronless reporter, ILRibo1:

GAATTCGAA AATTACTGA ATTAACAATG TCTTTATCTT CAAAGAT CTCC CATGTCTCTA
CTGGTGGTGG TGCTTCTTTGGAATTATTGG AAGGTAAGGA ATTGCCAGGT GTAGCTTTCT
TATCCGAAAAAGAAACTAGT ttaattaaCA TCTTTTA
CCCATACGATGTT CCTGACTATGCGGGCTATCC CTATGACGTC CCGGACTATG CAGGATCCTA
TCCATATGACGTTCCAGATT ACGCTGCTCA GTGctga ggc ggcccATTGA
ATTGAATTGAAATCGACCTA GCTAGC AGATCCTAAGGTACCTAATTGCCTAGA
AAACATGAGGATCACCCATGTCTGCAGGTCGACTCTAGAAAACATGAGGA
TCACCCATGTCTGCAGTATTCCCGGGTTCATTAGATCCTAAGGTACCTAA
TTGCCTAGAAAACATGAGGATCACCCATGTCTGCAGGTCGACTCTAGAAAA
CATGAGGATCACCCATGTCTGCAGTATTCCCGGGTTCATTAGATCCTAAG
GTACCTAATTGCCTAGAAAACATGAGGATCACCCATGTCTGCAGGTCGAC
TCCAGAAAACATGAGGATCACCCATGTCTGCAGTATTCCCGGGTTCATTA
GATCCCTAGGCTAGCATTCCGATAGATCAATTT TTTTCTTTCTCTTTCCCAT
CCTTTACGCTAAAATAAGTTTATTTTATTTTTGAATTTTTTATTTATATAC
GTATATATAGACTATTAT TTAT CT TTTAATGATTATTAAGATTTTATTAAACA
AAATTCGCTCCTCTTTAATGCCTTTATGCAGTTTTTTTTCCCATTCGATATTTCTATGTCGG
GTCAGCGTAT TTTAAGTTTA ATAACCGAA AATTCTGCGTTCGTTAGCGGCCGC

Sequences upstream of reporter genes (cloned into *Eco*R1 site of *CYC1* promoter in pMK121):

tetO7; *CYC1*; *ACT1*

-558 TGACCACACC TCTACCGGCA GATCAATTCC TCGATCGAGT TTACCACTCC
-508 CTATCAGTGA TAGAGAAAAG TGAAAGTCGA GTTTACCACT CCCTATCAGT
-458 GATAGAGAAA AGTGAAAGTC GAGTTTACCA CTCCCTATCA GTGATAGAGA
-408 AAAGTGAAAG TCGAGTTTAC CACTCCTCAG TGACTATAGA GAAAAGTGAA
-358 AGTCGAGTTT ACCACTCCCT ATCAGTGATA GAGAAAAGTG AAAGTCGAGT
-308 TTACCACTCC CTATCAGTGA TAGAGAAAAG TGAAAGTCGA GTTTACCACT
-258 CCCTATCAGT GATAGAGAAA AGTGAAAGTC GAGCTCGGTA CCCTATGGCA
-208 TGCATGTGCT CTGTATGTAT ATAAACTCT TGTTTTCTTC TTTTCTCTAA
-158 ATATTCTTTC CTTATACATT AGGTCCTTTG TAGCATAAAT TACTATACTT
-108 CTATAGACAC GCAAACACAA ATACACACAC TAAATTACCG GATCAATTCCG
-58 GGGGATCGAC GGTATCGATA AGCTTGATAT CGAATTCCGA AAATTTACTG

-8 AATTAACA¹AT G

HIS3 flanking regions for homologous recombination into reporter strains (via pMK121 or pMK123).

HIS-5' flank 5' – 3'

aaacgaaggcaaagatgacagagcagaaagccctagtaaagcgtattacaaatgaaccaagattcagattgcgatctctttaagggtggtcc
cctagcगतगगcactcgatcttccagaaaaagaggcagaagcagtagcagaacaggccacacaatcgcaagtgattaacgtccacacagg
tatagggtttctggaccatatgatacatgctctggccaagcattccggctggctgctaatacgttgagtgattggtgacttacacatagacgaccatc
acaccactgaagactgc

HIS 3' flank 5' – 3'

tttgcaaggctagcagaattaccctccacgttgattgtctgcgaggcaagaatgatcatcaccgtagtगगagtgcgttcaaggctcttgcggtg
ccataagagaagccacctcgcccaatggtaccaacgatgttccctccacaaagggtgttcttatgtagtगacaccgattatttaaagctgcagcata
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